بسم الله الرحمن الحسم

KULLIYYAH OF PHARMACY

NANOTECHNOLOGY FOR FABRICATION OF NANOPARTICLES AND NANOEMULSION

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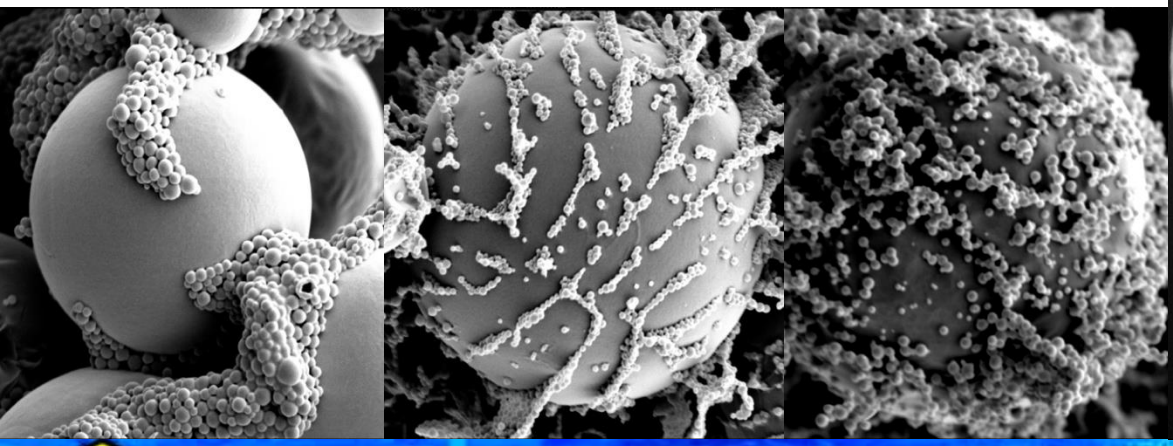
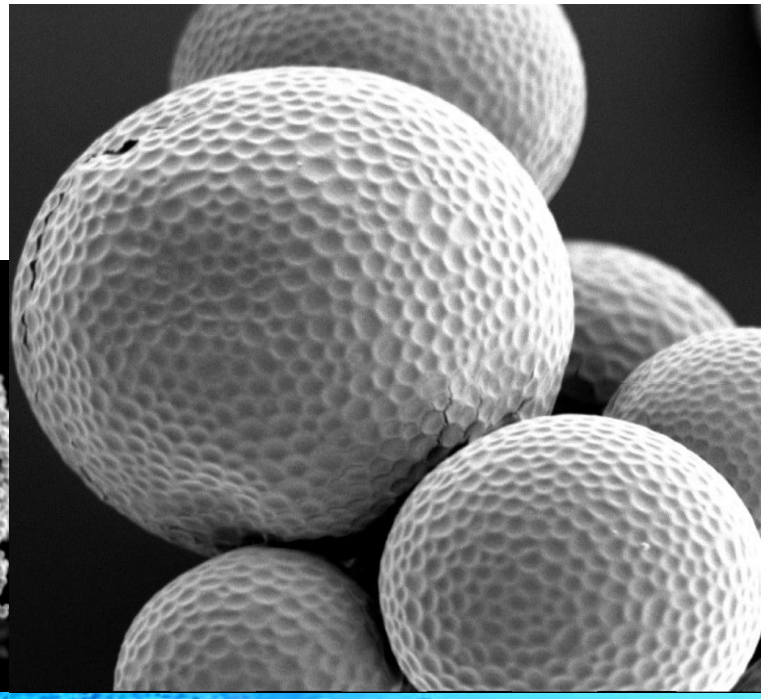
FARAHIDAH MOHAMED





Outline

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- 3 concepts
  - ✓ Design of Dosage Forms Using Nanotechnology
  - ✓ Biopharmaceutic & Biopharmaceutic Classification System
  - ✓ Fusion concept between contemporary and prophetic medicine
  - ✓ Summary of Discussion & Conclusion
- 2 strategies
  - ✓ Fabrication of plasmid DNA- Nigella sativa oil nanoparticles
  - ✓ Fabrication of Doxycycline – Nigella sativa nanoemulsion
  - ✓ Summary of Discussion & Conclusion

Design of Dosage Form

What is Nanotechnology in global context – based on ISO Technical meeting 2010:

“application of scientific knowledge to manipulate and control matter in the nanoscale range to make use of size- and structure-dependent properties and phenomea distict from those at smaller or larger scales”.

(Full acceptance and use of the ISO definition in the environmental, health, and safety (EHS) community has not yet been resolved) (Lovestam et al. 2010)

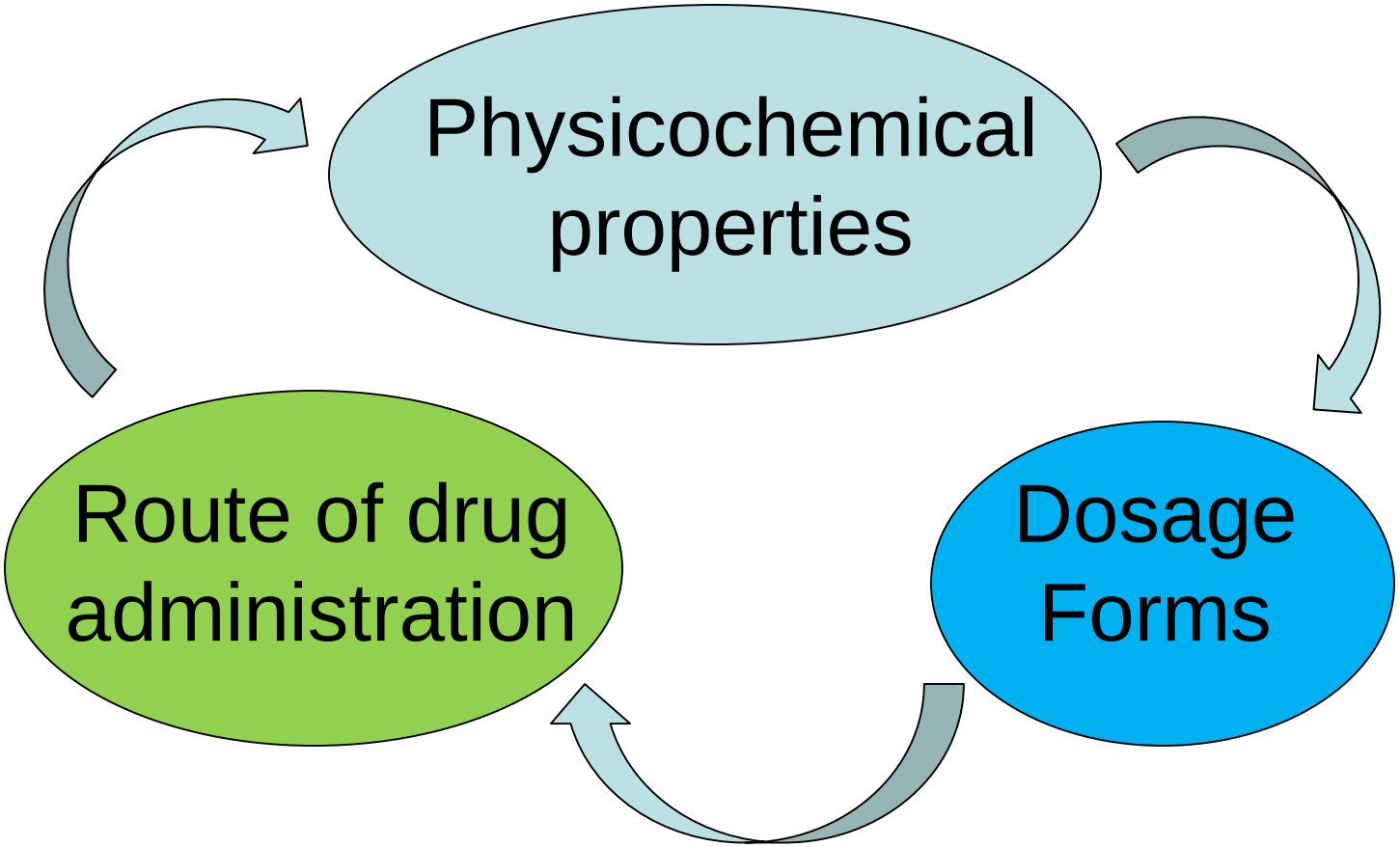
Design of Dosage Form

What is nanotechnology in the context of Drug Delivery System –

"science/engineering or technology that allows any raw materials (e.g small MW drugs, natural products, macromolecules) to become NANO-sized particles/crystals/powders".

What is NANO-sized particles = NANOparticles→ less than 300 to 100 nm (the size is chosen based on safety profile)

Concept #2.1
Biopharmaceutic



Rate & extent of absorption

= BIOAVAILABILITY

BCS – Biopharmaceutics Classification System

- Class I – High Permeability & High Solubility — HIGHEST ORAL F
- Class II – High Permeability & Low Solubility — HIGHER ORAL F
- Class III – Low Permeability & High Solubility
- Class IV – Low Permeability & Low Solubility

Contemporary medicine

+

Prophetic medicine

Novel Dosage Form

Fusion medicine

FUSION CONCEPT

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Novel Dosage Form

Nanoparticles

- DNA + Nigella sativa oil

Novel Dosage Form

Nanoemulsion

- Doxycycline hyclate + Nigella sativa oil

Contemporary + prophetic/herbal

Fabrication of Plasmid DNA + Nigella sativa Nanoparticles

Fabrication of nanoparticles

Physicochemical properties

Plasmid DNA

- labile
- Negatively charged
- ~5 kilo base pair
- Water-soluble / hydrophilic
- Precipitate in ethanol
- Size ~1-2 nm

Fabrication of nanoparticles

Physicochemical properties

Nigella sativa oil

- Has multiple compounds (~50% fatty acids + ~40% sterols + 10% tocopherol)
- Lypophilic / oil
- Soluble in organic solvent
- Surface tension ~30mN/m

Water surface tension ~72mN/m

Inspiration on selection of Habbatus Sawda

- Al-Bukhari: Related by Aishah (Allah bless her) that the Prophet had said: the black seed cures everything except death.

Mentioned in Ancient Medical Manuscripts

Ibn Sina in Canon of Medicine

Sahih Muslim: Book 26 Kitab As-Salam, Number 5489

Sahih Bukhari vol. 7 book 71, No 592).

Old testament : Isaiah 28:25,27

Tibb An-Nabawi Ibnu Al-Jauziyah

Tayyibul Ihsan Fi Tibb Al-Insan of Syeikh Ahmad Al-Fatani

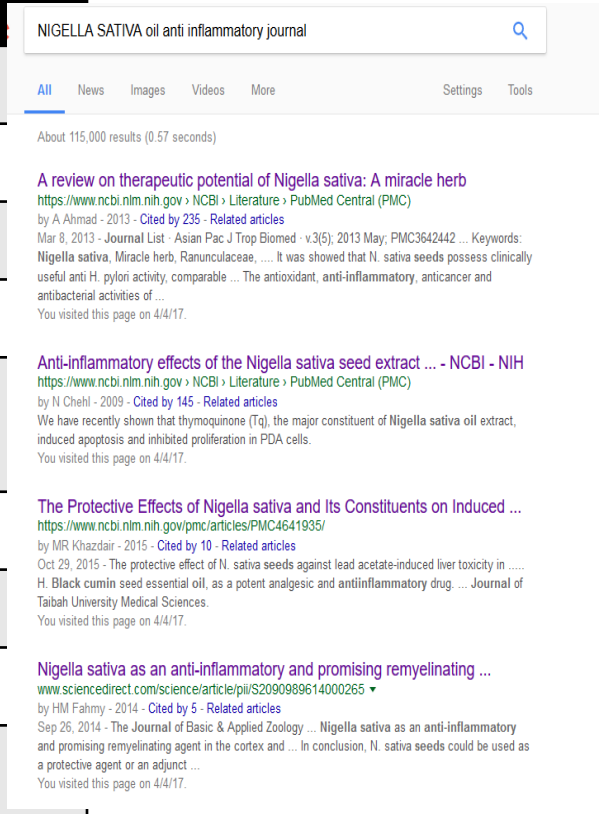
Kalonji is known around the world by many names because of its ancient popular history and medicinal value viz: black caraway, roman coriander, carvi (french), chwarzkummel (german), kalonji (hindi/urdu), kezah(hebrew) chernushka (russian), corek-out (turkish), habbat-albarakah or habbatus-sauda (Arabic) siyah daneh (Persian) etc.

Inspiration on selection of Habbatus Sawda

KULLIYYAH OF PHARMACY

Contemporary Medical Manuscripts

Ingredients		Quantity
Total oil 28-36%	Linoleic acid	40.3-58 %
	Oleic acid	18-28 %
	Steric acid	2.6-3.1 %
	Palimitic acid	10-12%
Tocopherol (9-25mg/100g)	α-tocopherol	1.7-4 mg/100g
	β-tocopherol	4.9-18 mg/100g
	ɣ- tocopherol	1-18 mg/100g
Sterol (~2g/kg)	B -sitosterol	48-52%
	5-avenasterol+ campesterol+ stigmasterol+ 10 more sterols	~50%

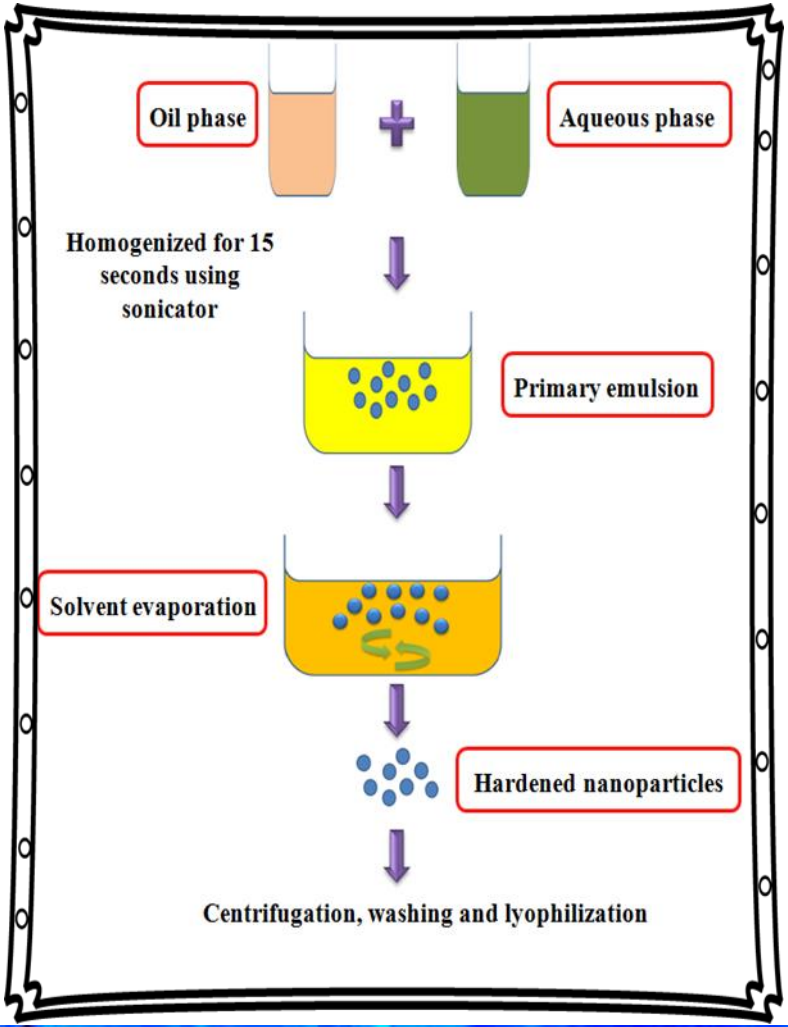


Contemporary Medical Manuscripts

Other constituents:

Thymoquinone, nigellicine, nigellidine, flavonoids, tannins iron, calcium etc.

FABRICATION OF NANOPARTICLES



OIL PHASE

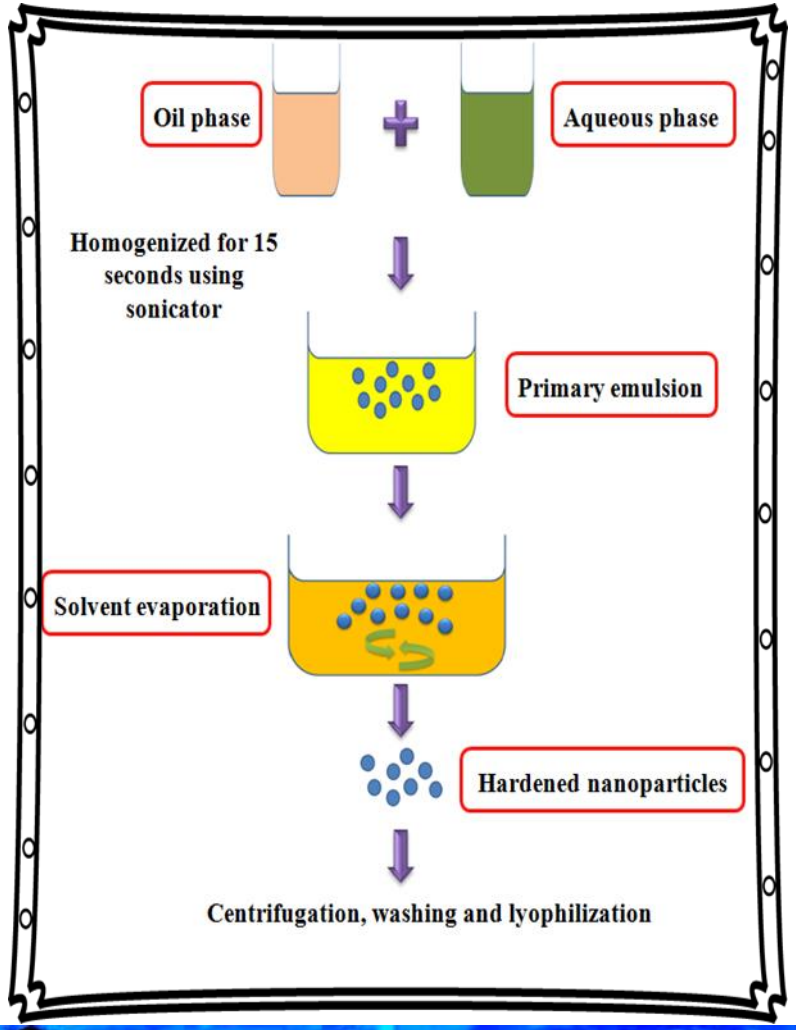
1.2% w/v PLGA (IV 0.2 dL/g; 14 kDa) + dichloromethane + ethyl acetate

+

1.2 % v/v Nigella sativa oil

FABRICATION OF NANOPARTICLES

KULLIYYAH OF PHARMACY



AQUEOUS PHASE

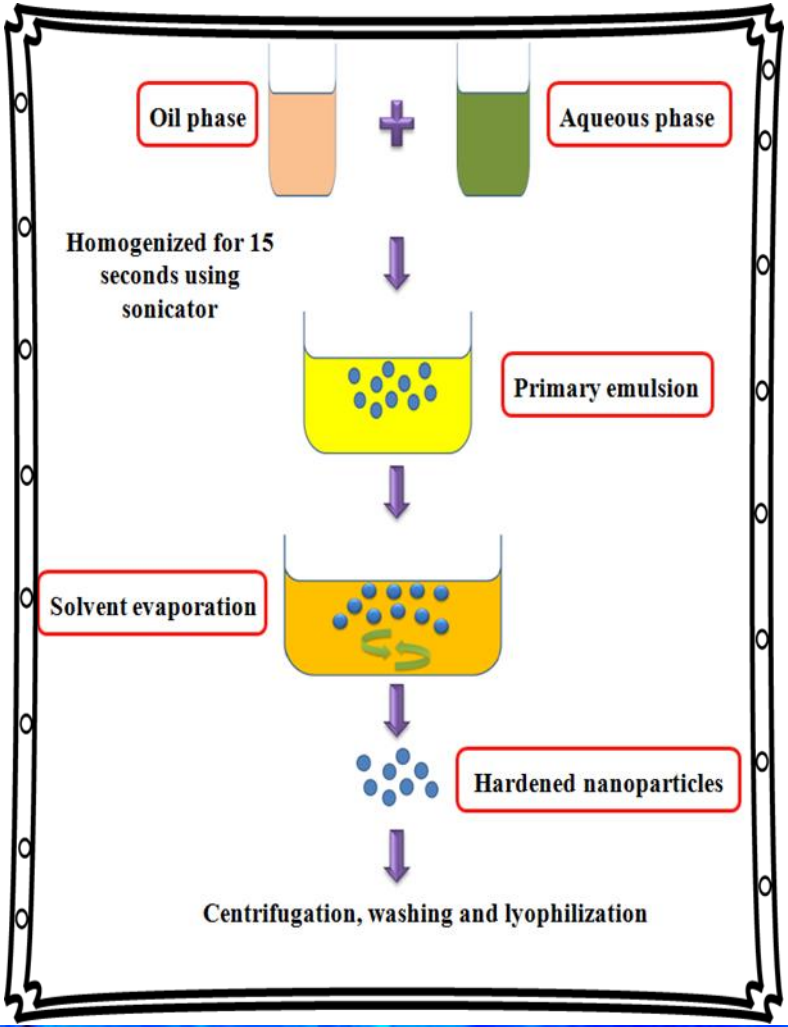
300 µg plasmid DNA (*Gaussia* luciferase vector) + 30 µl 2% CTAB

[redacted] + polyvinyl alcohol (PVA)

- Low Molecular weight chitosan
- Medium Molecular weight chitosan
- Without chitosan

FABRICATION OF NANOPARTICLES

KULLIYYAH OF PHARMACY



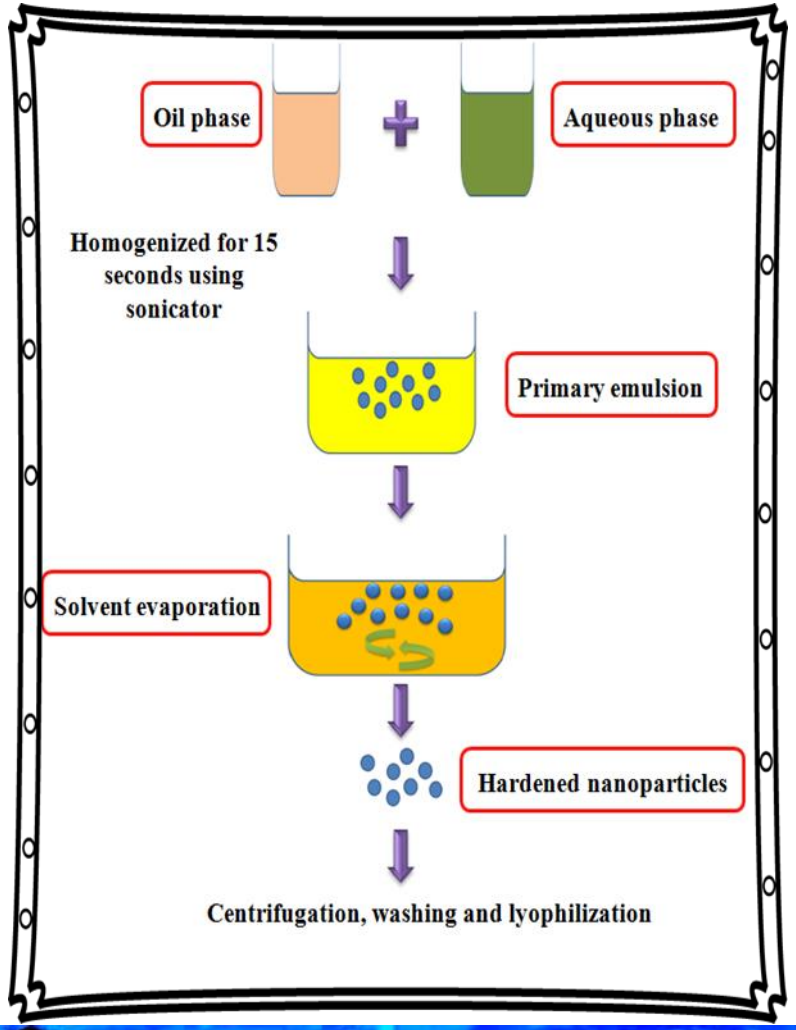
HARDENING TANK

DEIONISED WATER +

- Low Molecular weight chitosan
- Medium Molecular weight chitosan
- Without chitosan

FABRICATION OF NANOPARTICLES

KULLIYYAH OF PHARMACY



VARYING PARAMETERS

AQUEOUS PHASE

HARDENING TANK

Addition of Tween 80, High or Low MW chitosan

FABRICATION OF NANOPARTICLES

KULLIYYAH OF PHARMACY

Constant processing method, varying the ingredients

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FABRICATION OF NANOPARTICLES

KULLIYYAH OF PHARMACY

Constant processing method, varying the ingredients

Phase	Ingredients	pDNA-loaded PLGA/N. sativa nanoparticle formulations				
		BN	NA	NB	NC	ND
Oil	14 kDa (0. 2A dL/g) PLGA (mg)	30	30	30	30	30
	Dichloromethane (ml)	0.5	0.5	0.5	0.5	0.5
	Ethyl Acetate (ml)	2	2	2	2	2
	pDNA-CTAB (mg)	0.3	0.3	0.3	0.3	0.3
	N. sativa Oil (ml)	-	0.03	0.03	0.03	0.03
Aqueous	PVA 1% w/v (ml)	3	3	3	3	3
	Deionized water (ml)	6	6	5.64	4.2	4.2
	Tween 80 1% (v/v) (ml)	-	-	0.36	-	-
	LMW chitosan 2% (w/v) (ml)	-	-	-	1.8	-
	MMW chitosan 2% (w/v) (ml)	-	-	-	-	1.8
Hardening tank	Deionized water (ml)	36	36	34.6	33	33
	Tween 80 1% (v/v) (ml)	-	-	1.4	-	-
	LMW chitosan 2% (w/v) (ml)	-	-	-	3	-
	MMW chitosan 2% (w/v) (ml)	-	-	-	-	3

FABRICATION OF NANOPARTICLES

Characterisation

Varying media for particle sizing

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Characterisation

Surface morphology by Field Emission SEM

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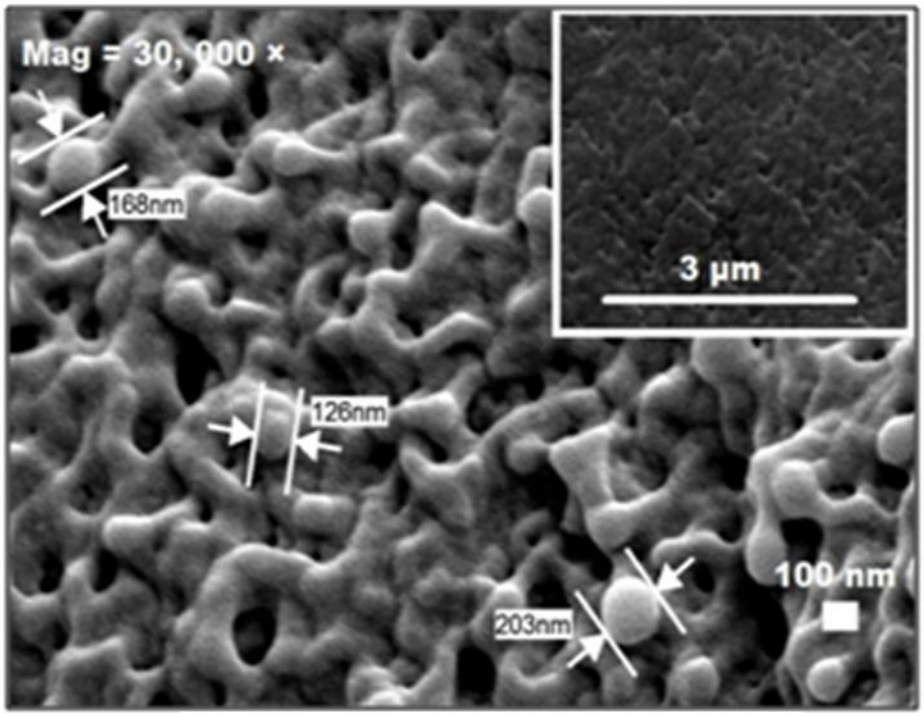
FABRICATION OF NANOPARTICLES

Characterisation

Surface morphology by Field Emission SEM

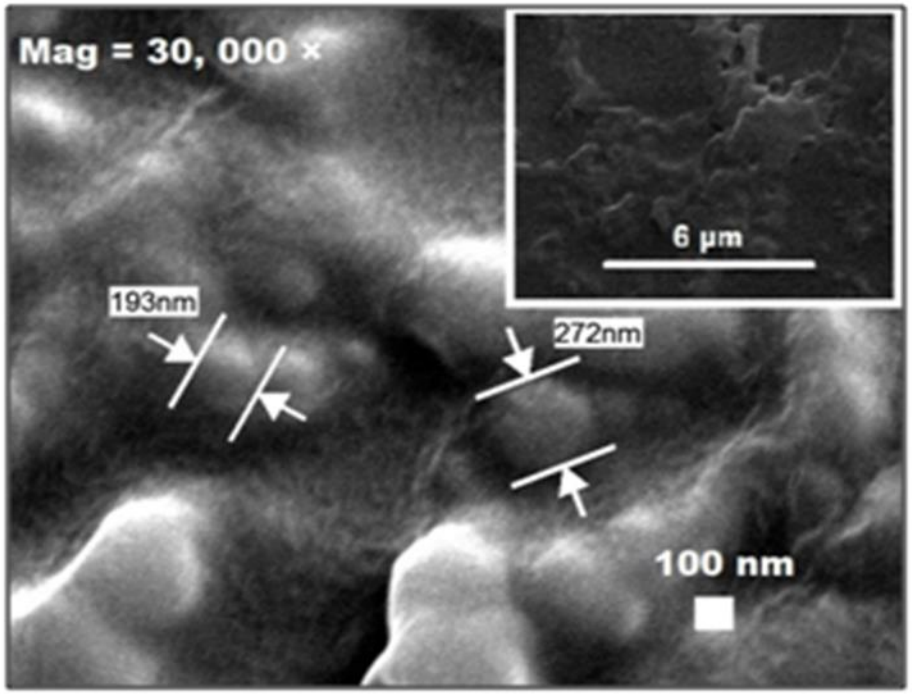


Blank Nanoparticles

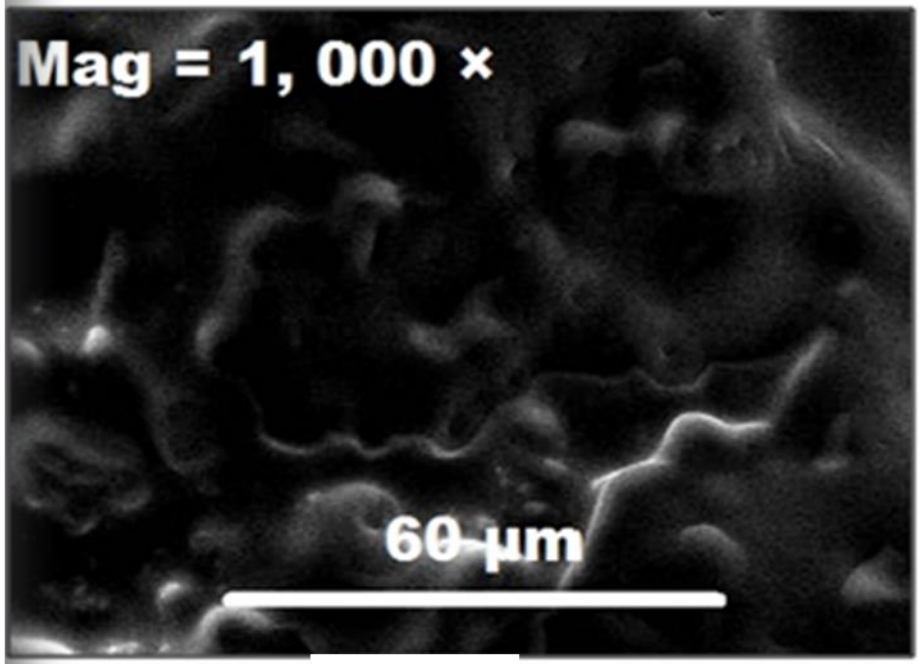
FABRICATION OF NANOPARTICLES

Characterisation

Surface morphology by Field Emission SEM



NA

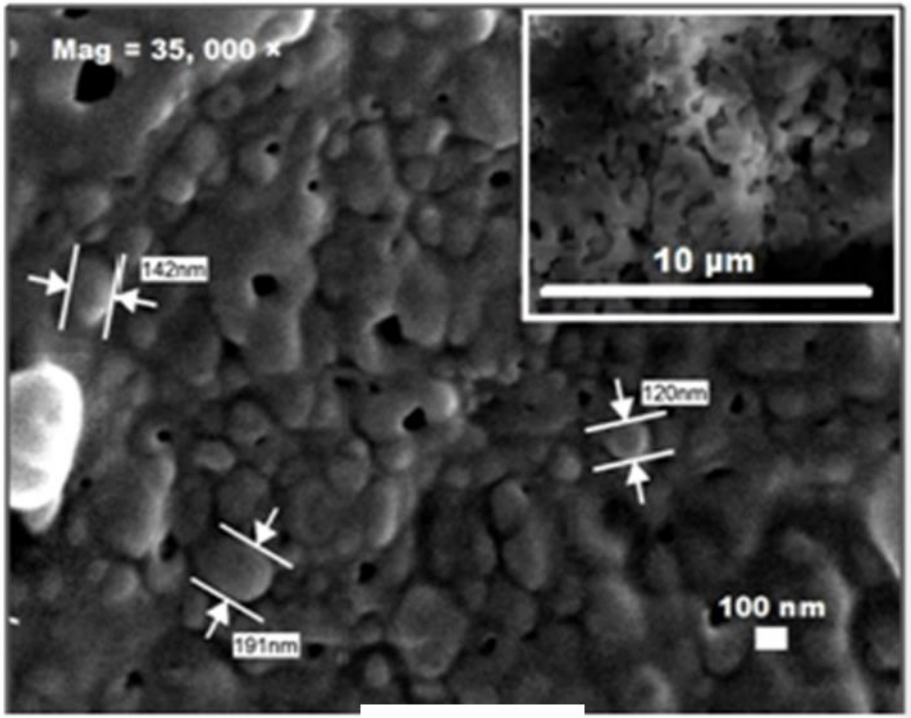


NB

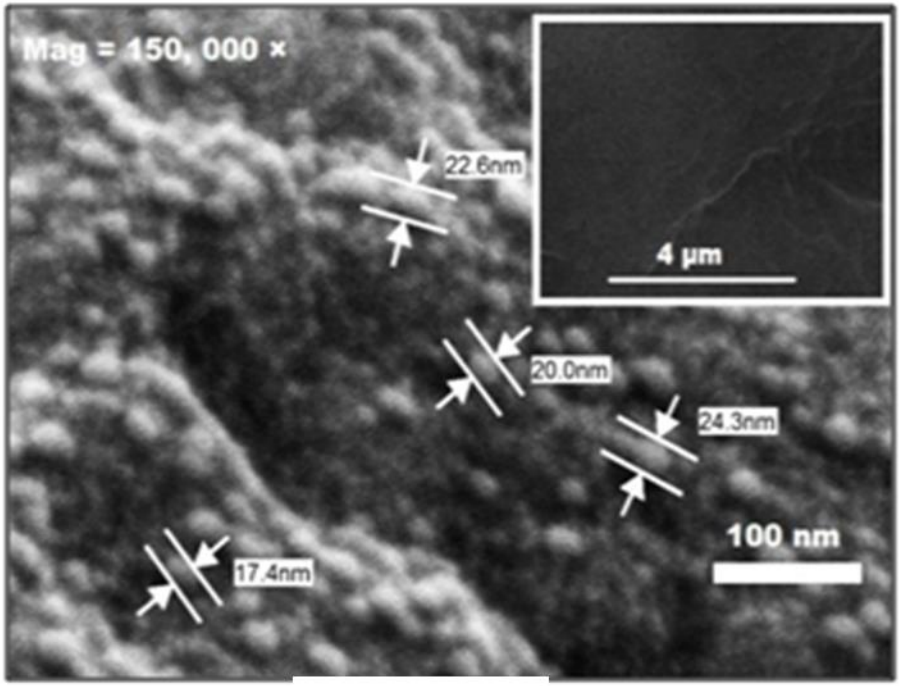
FABRICATION OF NANOPARTICLES

Characterisation

Surface morphology by Field Emission SEM



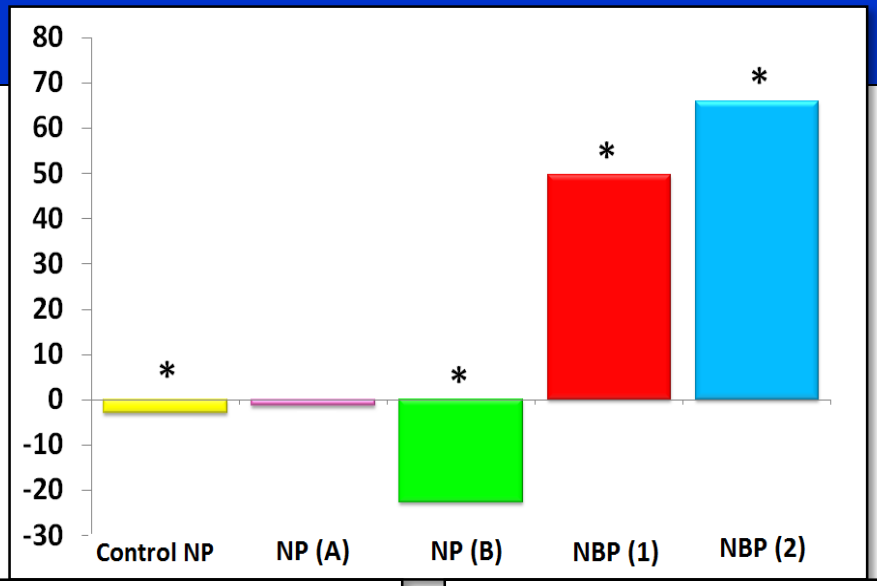
NC

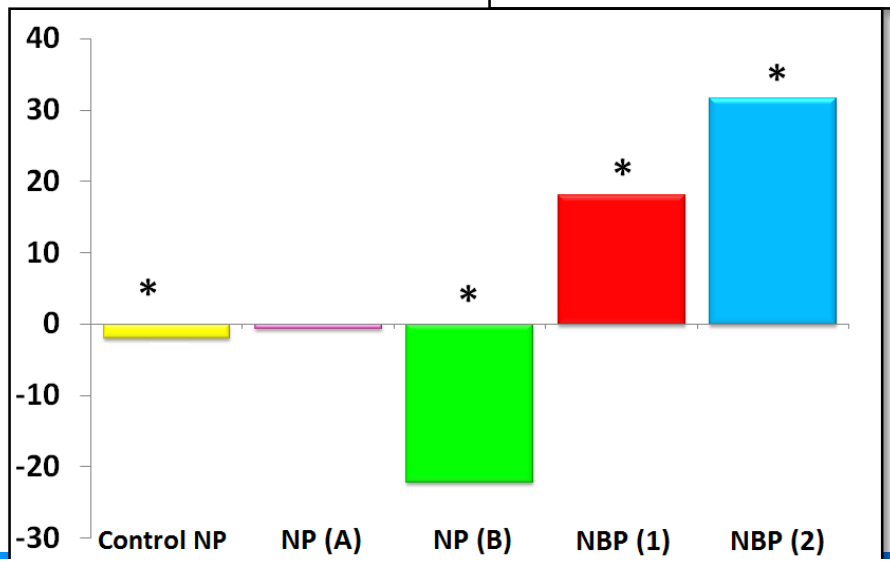


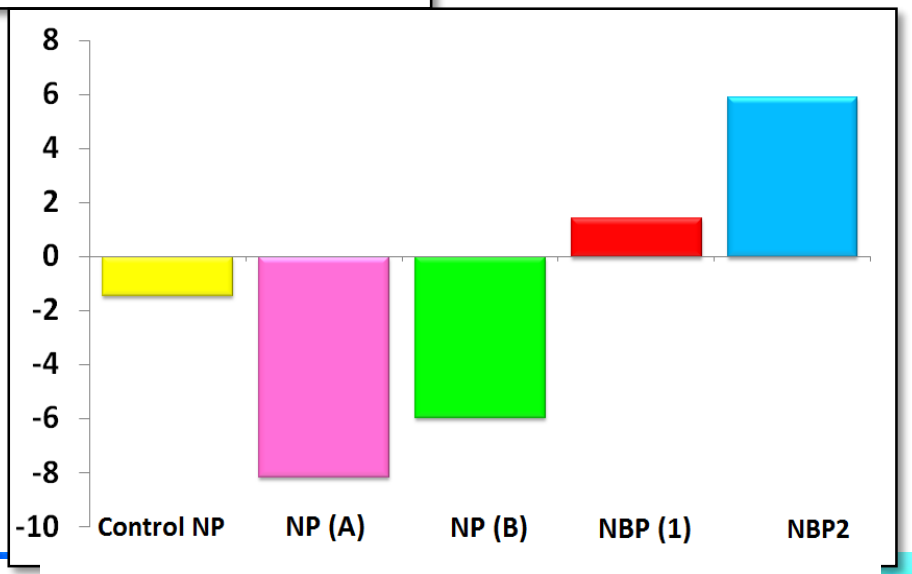
ND

Zeta potential

. on

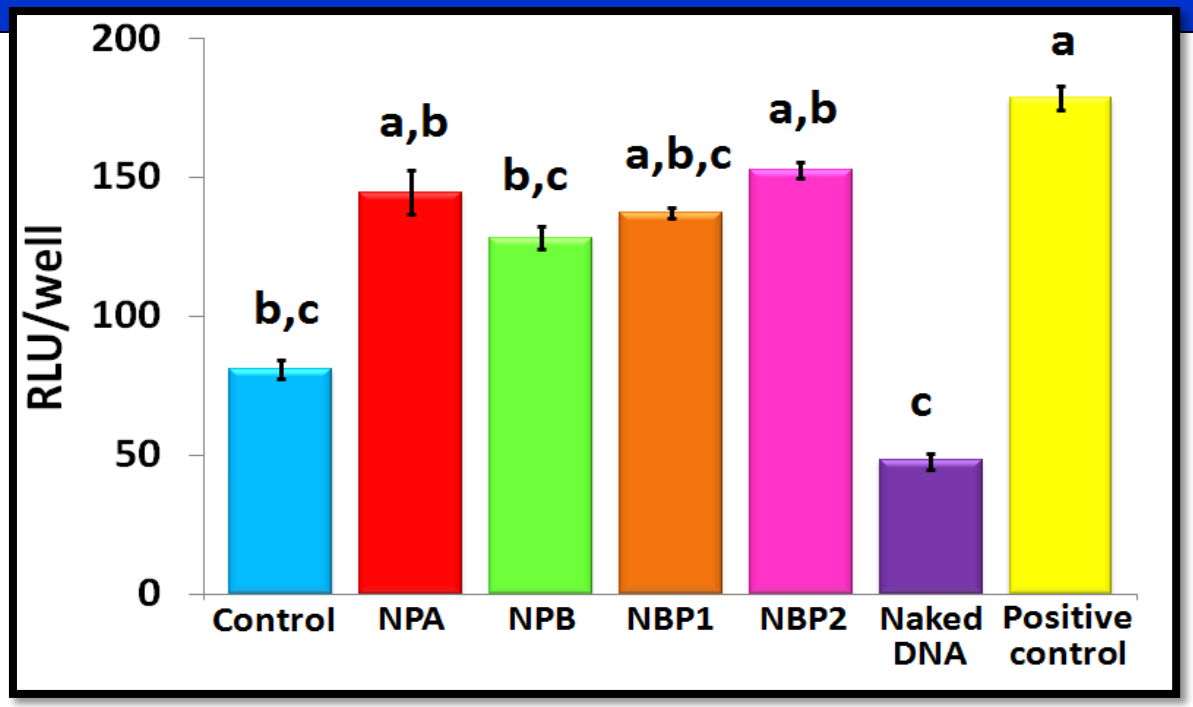






. yophilized suspended in the .

Transfection efficiency



❖he transfection could be seen in cells loaded with ., . . and . . having amount equivalent to .µg p. . . gave no significant difference compared to .a cells transfected with the positive control (.enejuice: . . :.. µl:.. µg) and negative control (.µg p. . .

❖f all, the cells loaded with chitosan bearing nanoparticle, . . which consisted of medium molecular weight of chitosan successfully transfected .a cells and gave pronounced gene expression.

Characterisation

N.Sativa effect on neurite outgrowth of Neuro2a cells

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ample	onc. (µg/ml)	.	.	.	.
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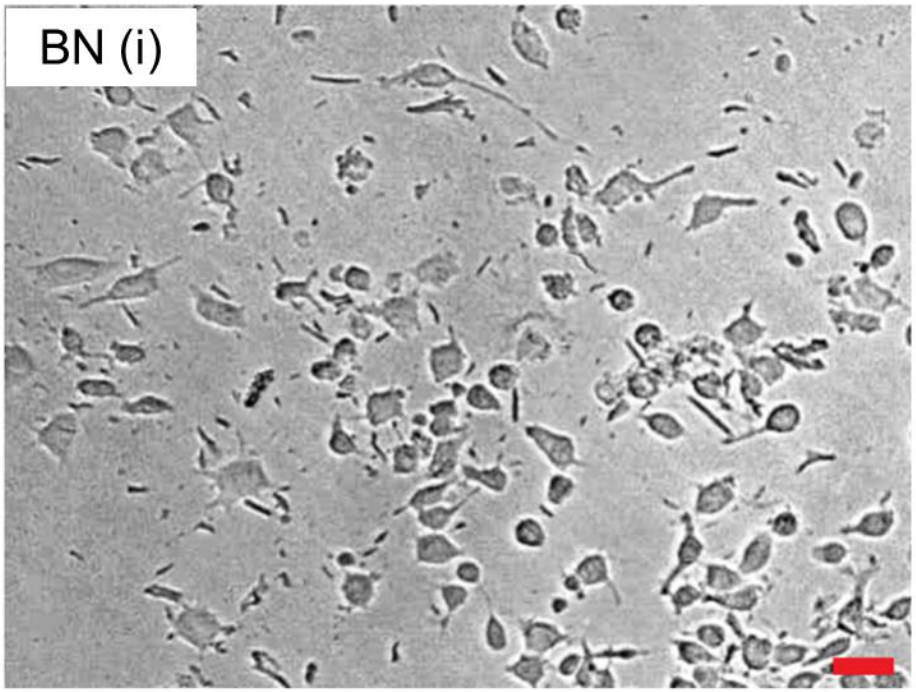
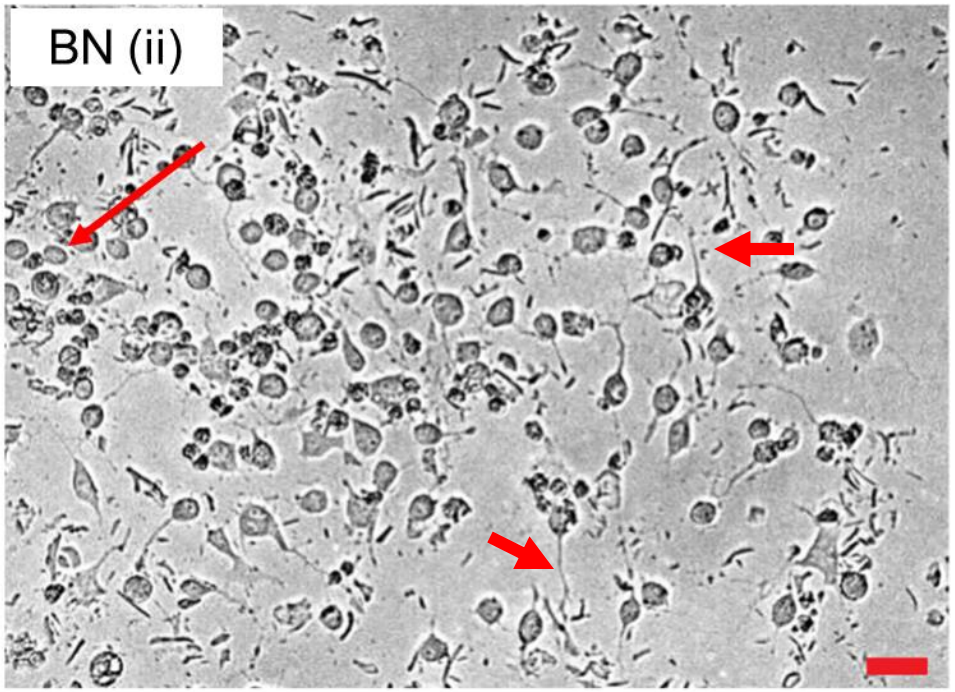
. engths (µm)

N.Sativa effect on neurite outgrowth of Neuro2a cells

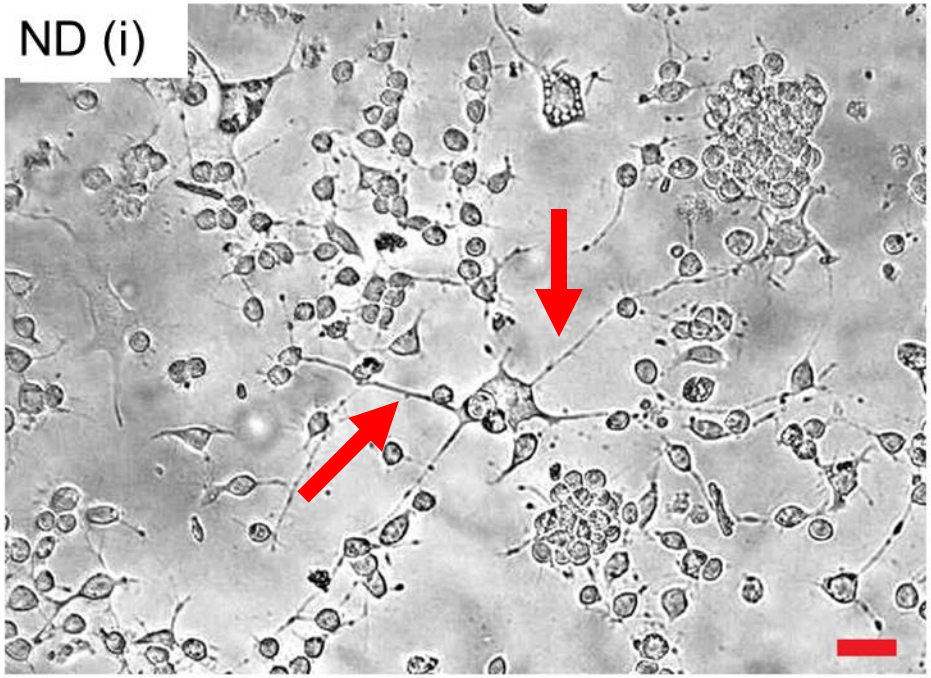


100 µg/ml of BN incubated for 24h

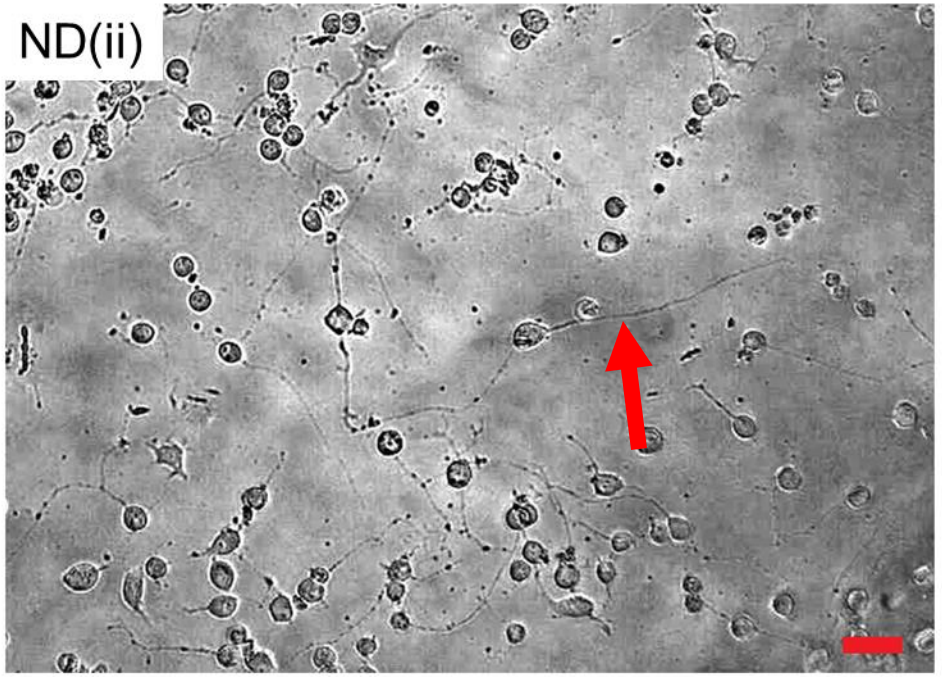


100 µg/ml of BN incubated for 96h

N.Sativa effect on neurite outgrowth of Neuro2a cells



100 µg/ml of ND incubated for 24h



100 µg/ml of ND incubated for 96h

Fabrication of Nanoparticles

Summary of Discussion & Conclusion

- Plasmid DNA was successfully co-encapsulated with N.sativa oil as nanoparticles
- This nanoparticles displayed ability to transfect (indicated pDNA remain functional) and able to induce neurite outgrowth (exerted by *N.sativa*) → probably potentially be used as neuroregenerative drug
- The fusion between contemporary medicine which is water-soluble (pDNA) and oily natural product cum prophetic medicine *(N.sativa)* showed enhanced gene transfection with another attribute i.e neuroregeneration

Fabrication of Doxycycline + Nigella sativa Nanoemulsion

Fabrication of Nanoemulsion

Physicochemical properties

Doxycycline

- Small molecular weight
- Water-soluble / hydrophilic
- Light-sensitive
- Commercially available in powder

Physicochemical properties

Nigella sativa oil

- Has multiple compounds (~50% fatty acids + ~40% sterols + 10% tocopherol)
- Lipophilic / oil
- Soluble in organic solvent
- Surface tension ~30mN/m

Water surface tension ~72mN/m

Fabrication of Nanoemulsion

Physicochemical properties

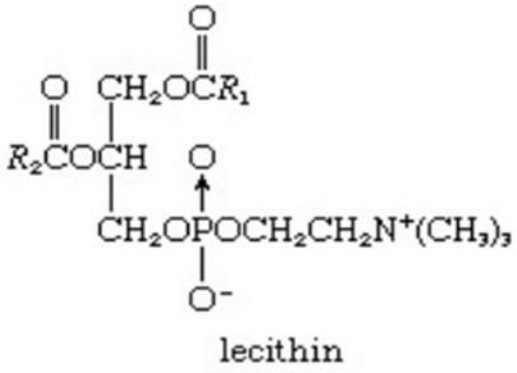
Lecithin

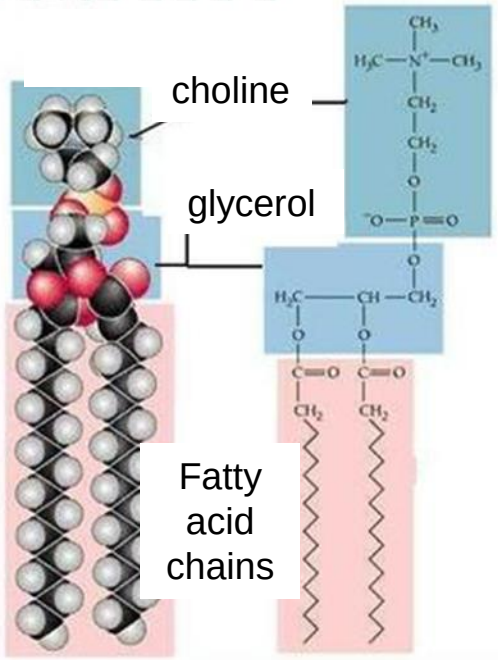
- a phospholipid molecule (phosphatidic acid, phosphatidylethanolamine, phosphatidylcholine and phosphatidylinositol)
- <36% Fats+fixed oil

Fabrication of Nanoemulsion

Physicochemical properties

Lecithin





A lecithin molecule has a nonpolar portion (fatty acid ester gp) and polar portion (phosphorylcholine) -→ amphiphilic molecule

- Composed of phosphoric acid, cholines, esters of glycerol, and two fatty acids
- The term ‘lecithin’ is also used for a mixture of phosphoglycerides containing principally lecithin and cephalin (phosphatidylethanol amine, phosphatidylinositol)

Preparation method:

Aqueous Phase

- Doxycycline
- Preservatives
- Dissolve using pneumatic stirrer

Preparation method:

Oil Phase

- Nigella sativa oil
- Eugenol
- Mix using pneumatic stirrer

Preparation method:

Nanoemulsifiers Phase

- Lecithin
- Co-surfactants + water
- Pulsative mixing using agitator (10, 20,10 mins; 50 rpm)

Fabrication of Nanoemulsion

Preparation method:

Nanoemulsification

- Aqueous and oil phases added into nanoemulsifier phase
- Combined technique of agitator (30rpm) + hamogenizer (3000rpm) for 30min
- Equipment: Vacuum Homogeniser

Preparation method:

Nanoemulsification

Vacuum Homogeneous Mixer

Strategy #2
Fabrication of Nanoemulsion

Characterisation

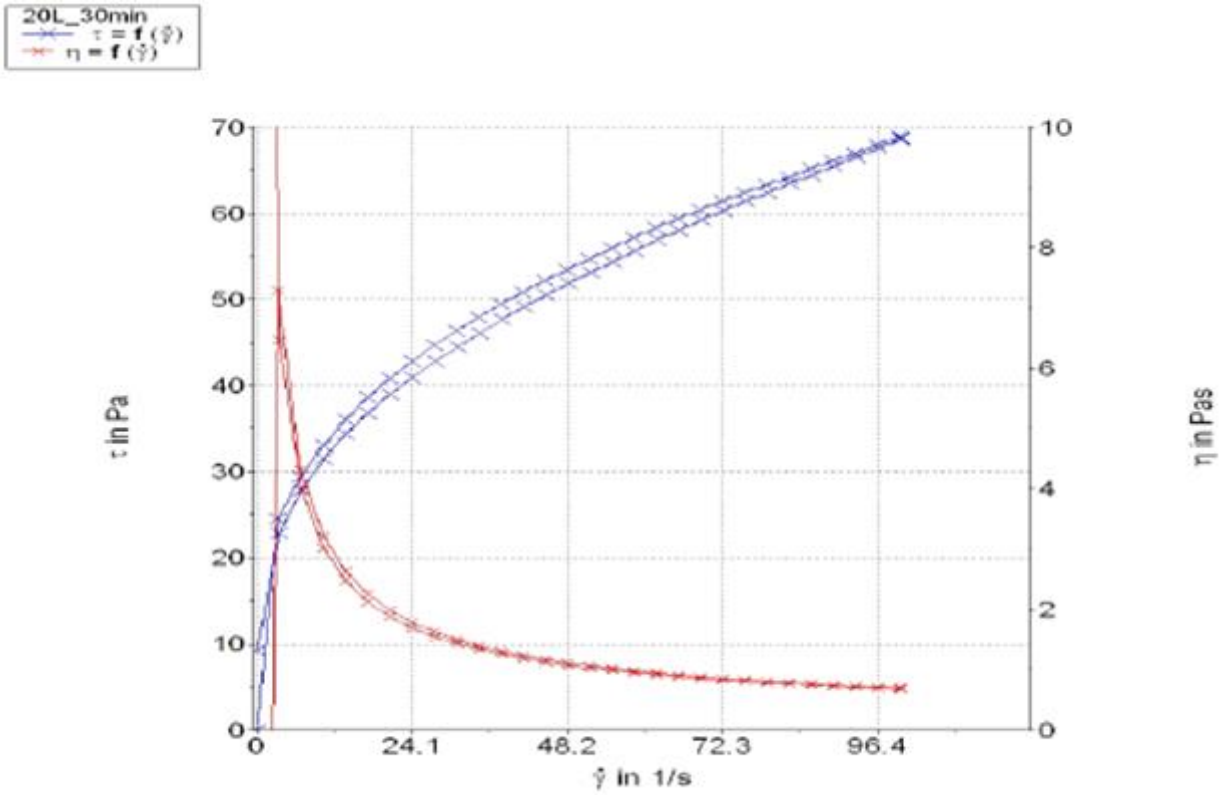
Droplet size, PDI, Zeta potential

Formula	Droplet size (nm)	PDI	Zeta potential (mV)
19% lecithin	288	0.427	-69.2
23% lecithin	235	0.389	-63.3

Strategy #2
Fabrication of Nanoemulsion

Characterisation

Rheological properties



19% Lecithin

Viscosity 3.22 Pa.s

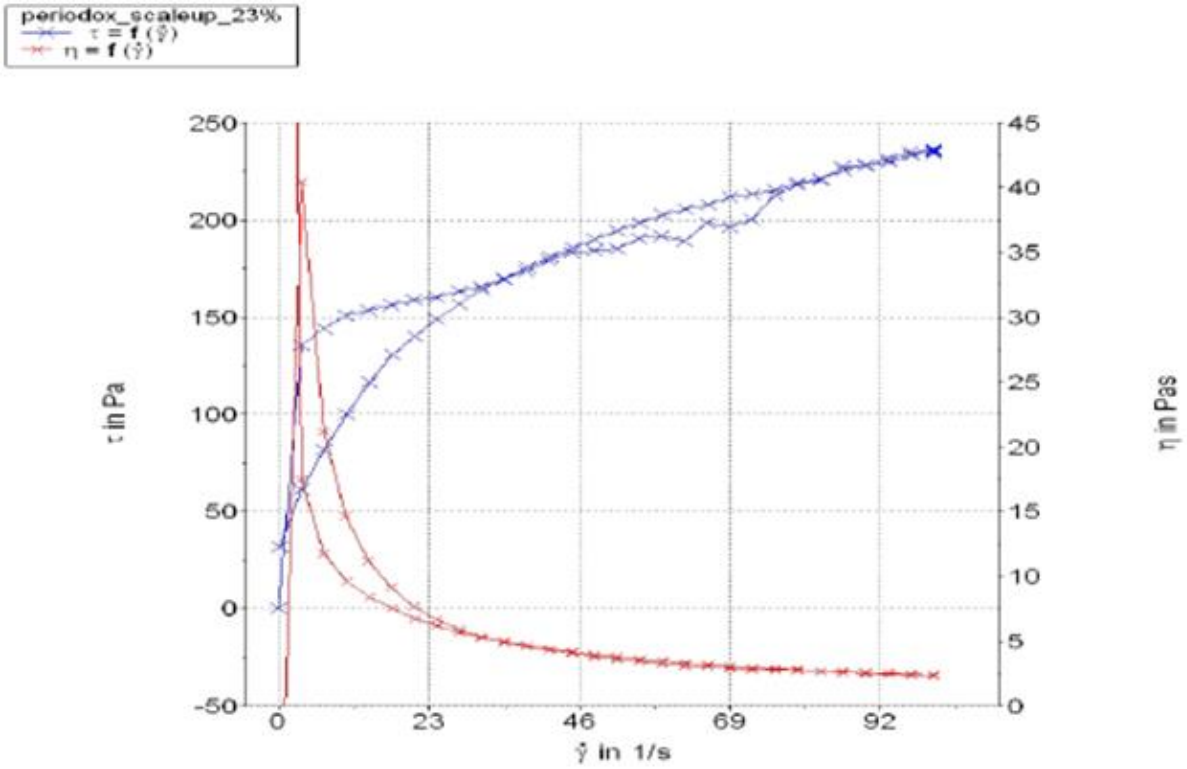
Thixotropy 124.4 Pa.s

Thixotropy *– broken down with time when sheared; rebuilds when left to rest*

Strategy #2
Fabrication of Nanoemulsion

Characterisation

Rheological properties



23% Lecithin

Viscosity 14.68 Pa.s

Thixotropy 650.2 Pa.s

Thixotropy *– broken down with time when sheared; rebuilds when left to rest*

Strategy #2
Fabrication of Nanoemulsion

Summary of Discussion & Conclusion

- Due to amphiphilic structure, lecithin with the addition of suitable co-surfactants can yield a stable nanoemulsion
- Nanoemulsion of doxycycline + Nigella sativa oil displayed a non-Newtonian, thixotropic fluid → suitable for intended use as locally applied antibiotic for periodontitis
- The fusion between contemporary medicine which is water-soluble API (doxycycline) and oily natural product cum prophetic medicine *(N.sativa)* is postulated to improve doxycycline efficacy due to improved 'BCS' classes, from BCS Class III to BCS Class I


The End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&
A